Reflections on biochemistry

DNA

Early nucleic acid chemistry

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with A Serg

The contemporary biochemist, perusing the publications of the past century, may easily be misled into some arrogance when sorting out the erroneous data and leads, the slow progress and the primitive reports of that early period. However, when recalling the limited techniques and the lack of facilities at the early stages of biochemistry, he will change his mind. The difficulties encountered by the investigators of nucleic acid structure were many. To begin with, it was difficult to ascertain the degree of uniformity of the starting material. There were no established methods for studying macromolecules, and no guidelines for the isolation of structural units. Fortunately for the identification of new compounds, reference material or closely related substances had already been synthesized by organic chemists in experiments that were usually unrelated to nucleic acid problems. Much information on purines already existed, and some pyrimidines had been synthesized. Emil Fischer's work on monosaccharides provided the basic information for the identification of ribose and deoxyribose. Thus, nucleic acid research of that period owes much to organic chemistry.

Beginnings

The history of nucleic acid research begins with Friedrich Miescher's search for chromatin in pus. Miescher was trained in Basel, in the laboratory of his uncle, the renowned anatomist Wilhelm His, Sr. After receiving his medical degree in 1869, he joined F. Hoppe-Seyler in Tuebingen. Extraction of soiled bandages from infected wounds led to the isolation of material that he considered to be a protein, more acid in nature than any earlier known protein. Miescher's publication was delayed, and when it appeared two years later in an irregular collection, *Hoppe-Seyler's Medizinisch-Chemische Untersuchungen*, his mentor had added two papers of other co-workers in which similar acid protein material from different sources was reported[1].

This delay in publication was not unusual for the period. The practice of publishing research findings as they occurred was unknown prior to the establishment of the *Zeitschrift fuer Physiologische Chemie* in 1877. Its founder[2], Felix Hoppe-Seyler, along with T. Schwann, was a pupil of the physiologist Johannes Mueller. At the University of Tuebingen, which was established around 1500, Hoppe-Seyler had research quarters in the picturesque old castle; a tablet at the entrance to a side wing commemorates his activity there from 1862 to 1872. Thereafter, he accepted a position at the University of Strasbourg.

Miescher returned to Basel in 1870, to start his academic career and to continue the investigation of chromatin. His study on 'Protamine, a New Organic Base from Salmon Sperm'[3] achieved better uniformity of source material, extraction technique, results and speed of publication.

> Nuclein was found to be the principal fraction (48.7%); it is an albuminoid substance of acid property, rich in P (9.6%), and free of sulfur. Several similar compounds have been isolated earlier from various sources in an impure state. Now, for the first time, the possibility of its preparation in the pure state has been realized. Nuclein is combined with protamine; a mixture of several basic components may hardly be assumed.

The latter remark pertains to protamine-like compounds; Miescher was not aware of the presence of purines and pyrimidines. However, on his suggestion Jules Piccard*, the head of the chemistry department in Basel, did more work on nuclein and found 6–8% of the previously known purine bases guanine and hypoxanthine (sarkin) as components[4]. He suggested that 'the presence of such significant quantities of these rare compounds in a readily accessible material may be of some interest to chemists and physiologists'. The quality and speed of Piccard's publication suggests that he would have isolated all nucleic acid bases in the course of a few years, had he only continued the investigation of nuclein. Instead this task took several decades in the hands of others.

Slow progress

The term 'nucleic acid' was used first in 1889 by R. Altmann[5], who studied the phosphate-containing material from thymus, egg yolk and salmon sperm; his results confirmed Miescher's observations. However, the term 'nuclein' prevailed until the turn of the century. No attempt was made by Altmann to confirm Piccard's discovery of guanine. Likewise, A. Kossel in his numerous publications made only passing reference to Piccard's work. He may have felt that the latter had preempted his domain, because as late as in 1910, he wrote: 'Guanine has been known for some time in various animal tissues, and was found, for example, in the spermatozoa of salmon by Piccard, although indeed this investigator had no suspicion that it had any genetic relationship with nuclein' (Ref. 6, p. 396).

The recognition of two distinct types of nucleic acid, DNA and RNA, occurred about 25 years after Miescher's discovery, and another 40 years passed before the first biological function was described. During this early period, the limited amount of nucleic acid research was mainly in the hands of investigators with a medical background. Organic chemists at that time did not take much interest in these ill-defined, almost untractable compounds; in their own field, the methods of organic synthesis provided easy access to new substances that could be purified readily and often had great commercial value.

Neither Miescher (1844–1895) nor Hoppe-Seyler (1825–1895) contributed much to the biochemistry of 'nuclein' beyond their initial work. In 1871, Miescher was appointed to head the department of Physiology in Basel. He then devoted his efforts mainly to cytological problems, but this work was interrupted repeatedly by bouts of tuberculosis[7].

* Jules Piccard came from a renowned Swiss family of scientists; his twin sons, Jean-Felix and Auguste, later became famous for balloon excursions into the stratosphere and diving records by bathysphere to explore the depths of the oceans.

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A new approach

Biochemical investigations of nucleic acids came to be one of the main activities of another of Hoppe-Seyler's students, Albrecht Kossel (1853–1927). Heidelberg was the principal site of his academic career, and he attracted many co-workers and guest investigators to his laboratory. E. Kennaway[8] has given us a charming set of recollections of his sojourn with Geheimrat Kossel, and of the commotion and torchlight parade of the students when the award of the Nobel Prize to Kossel was announced in 1910. Kossel was probably the first investigator to surmise that nuclein may be involved in growth and differentiation[9]:

> The assumption that nuclein is a resource, at the expense of which a starving organism lives, has to be refuted on the basis of all experiments. The quantity of nuclein changes little, whether the organism is starving or not.

The most valid basis for this conclusion seems to have been Miescher's observation on salmon sperm, which is formed at the expense of tissue reserves; the fish does not take nutriment during the period of spawning. Kossel studied this problem with a few starving chickens, but the quantitation of nucleic acid by the methods of that time was rather uncertain.

Components identified

Progress in clarifying the structure and composition of nucleic acids was painfully slow. Guanine was known prior to its isolation from nuclein by Piccard[4]; it had been discovered already in 1846 by Unger[10] in guano, the bird excreta imported as fertilizer to Europe from the South American west coast. Guanosine was isolated by Schulze and Bosshard in 1886 from plant material[11]. A relation to nucleic acids was not suspected, but the identity was established in 1910 by comparison with the nucleoside that Levene had obtained from guanylic acid[12]. Adenine was isolated from thymus gland by Kossel[13]; he derived the name from the Greek term for gland: aden, adenos. The name purine was coined by Emil Fischer[14] to indicate the unaltered, pure nature of the basic ring system. Kossel and Neumann[15] discovered thymine and came to the conclusion that a carbohydrate group is present in thymus nucleic acid. Kossel's co-workers, Ascoli and Steudel, discovered cytosine and uracil. Pinner[16] synthesized pyrimidine and suggested its name in analogy to pyridine. Sometimes, degradation products of nucleic acid bases were obtained and, for a period, hypoxanthine and xanthine were believed to be nucleic acid components. Also, a variety of nucleic acids was assumed, each having only one kind of a base. It is impossible to enumerate all the misconceptions and the work necessary to undo them. Review articles were not customary at that time, but the Nobel Lecture of A. Kossel in 1910 gives an idea of all the vagaries of early nucleic acid biochemistry[6].

Ribose and deoxyribose were the last principal nucleic acid components to be identified. The investigations of Emil Fischer and his school on carbohydrates provided the fundamental information[17]. Xylose and arabinose were known then as naturally occurring pentoses, and Fischer projected the configuration of the other two pentoses and suggested the names lyxose and ribose by rearrangement of some of the letters of xylose and arabinose, respectively[18]. How different would the development of nucleic acid biochemistry have been, if Fischer had made it one of his main enterprises!

The isolation of the carbohydrate of pentose nucleic acid was tried repeatedly, and its identity with arabinose, xylose, and lyxose was suggested by various investigators. Success in the identification of ribose[19] and deoxyribose[20,21] in 1909 and 1930, respectively, was achieved by P. A. Levene and his co-workers, mainly W. A. Jacobs, E. S. London, T. Mori, and S. R. Tipson. In both instances, the isolation of the nucleosides was a pre-requisite to provide the starting material. Here again, Levene did the pioneering work; the term nucleoside was coined by him for the reason that they link carbohydrate in a glycosidic union to the nucleic acid bases. His work with Tipson and with Stiller also led to the recognition of the furanoid structure and of positions 3 and 5 of the pentoses as the sites of esterification of phosphoric acid.

Levene's numerous contributions were summarized in collaboration with L. W. Bass in 1931, in *Nucleic Acids*[24], the first monograph of consequence covering the entire field, with emphasis on the chemistry of nucleic acid constituents; of necessity, biological data were minimal and altogether speculative at that time. In all, Levene has done far more for chemical nucleic acid research than any of his predecessors[25]. His work is often underestimated by biochemists and biologists of a more recent period, who did not forgive him his erroneous conept of the 'tetranucleotide structure' and his reservation about a possible macromolecular structure of nucleic acids. Several other leading specialists of that period, including Steudel and Feulgen, likewise favored the concept of a tetranucleotide structure, and nobody contested it. In his famous monograph (1931), Levene devoted only a few pages to 'Nucleic Acids of a Higher Order'. He summarized his opinion[24]:

> Thus, in conclusion, it must be admitted that judgement as to the existence of nucleic acids of a higher order should be postponed until the work is repeated on a larger scale. On the other hand, the presence of a ribopolynucleotide in the animal tissues must now be regarded as well-established.

In the late 1930s, however, Levene accepted the macromolecular structure of all nucleic acids, which by then had been firmly established by ultracentrifugation and dialysis experiments. Still, the repetitive occurrence of tetranucleotide units in these macromolecules persisted in the mind of many investigators of that period. J. A. Witkowski[26] recently has reviewed the reasons for such simplifying concepts of macromolecular structure of nucleic acids as well as proteins. Numerology, psychology and inadequate analytical data played a role.

Organic chemistry also played a key role in other developments of nucleic acid research. As described recently by J. Brachet[27], the production of special stains and the development of specific color reactions greatly aided progress in nucleic acid cytochemistry. Thus, during the second quarter of this century, cell biologists showed an increasing interest in nucleic acids and lifted them from the status of biochemical oddities. A fortunate interplay among scientific disciplines resulted.

The beginnings of a new era

The first unequivocal demonstration of a specific biological activity of DNA was provided in 1944, by O. T. Avery, C. M. MacLeod and M. McCarty[28]. They succeeded in demonstrating that the transforming principle isolated from smooth cultures of pathogenic *Pneumococcus* is a specific deoxyribonucleic acid. Recognition of their results, however, was not immediate. As described by M. McCarty[29], one of the first comprehensive presentations of the data was given by him at an exclusive meeting of top scientists, among them seven Nobel Laureates, and a small group of younger scientists. Unfortunately, the proceedings of this conference in 1945, at the resort of Hershey, Pennsylvania, were not published. I remember the excellent presentation given by Maclyn McCarty; unfortunately, it did not arouse much excitement at the time. One of the Nestors of the group, Linderstrøm-Lang, restricted his summarizing remarks about progress essentially to the mysteries of proteins. He bemoaned their complexity and stated that the primary structure of proteins probably never could be resolved, and that synthetic efforts at best would lead to caricatures of the cellular products. Proteins preoccupied the affection of most investigators at that time to such an extent that their surmized role as carriers of genetic information was not readily abandoned[29]. With some delay, however, the DNA experiments were extended, and other transformations were found. DNA became very popular.

The effects of E. Chargaff to elucidate the base composition of DNA from various species led to the recognition of individuality and of the A–T and G–C equivalence; from there it was only a short step to the double helix. The recognition of various kinds of RNA was not long in coming; H. G. Khorana synthesized polynucleotides, and the establishment of the genetic code by M. W. Nirenberg was the crowning event.

The limelight now shifted to the ever increasing studies of the biological functions of DNA and RNA. However, chemistry continued to provide valuable contributions. The most important – now historical – account of this transition period is the compendium on *Nucleic Acids, Chemistry and Biology*, edited by E. Chargaff and J. N. Davidson[30]. In its 46 chapters nearly all contributors to the progress of that period are represented. A similar effort now (three decades later) would involve many hundred contributors and result in a treatise filling a small library.

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